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The effects of sewage sludge on grassland euedaphic and hemiedaphic collembolan populations

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Summary. The application of sewage sludge to agricultural land is thought to provide a cheap and efficient method of sludge disposal. The positive aspect of recycling nutrients may, however, be counteracted by the risk of contamination by heavy metals that are frequently present in sludge. Collembola live in close association with the soil flora and fauna and may therefore be particularly susceptible to metal pollution. Effects of sewage sludge on euedaphic and hemiedaphic Collembola in grassland were assessed using litterbags as a sampling method. Small plot field trials were used to determine the effects of five different treatments: uncontaminated (i.e. low levels of heavy metals) digested sludge; uncontaminated undigested sludge; zinc-rich digested sludge; copper-rich undigested sludge and no sludge control. Although the application of sludge (irrespective of type) did not influence the overall abundance of Collembola in the study area, significant differences were found at the species level. *Isotoma anglicana* Lubbock was more abundant in plots receiving zinc and copper-rich sludge than in plots receiving uncontaminated sludge (digested or undigested) or no sludge, while *Mesaphorura* spp. were less abundant in zinc and copper-rich plots than in uncontaminated sludge or control plots. *Neelus minimus* Willem occurred in higher numbers in copper-rich plots but in lower numbers in zinc-rich plots when compared to uncontaminated plots. Seasonal and successional effects were also found and, for most species, these were more pronounced than the effects of sludge treatment. *Lepidocyrtus cyaneus* Tullberg, *Heteromurus nitidis* (Templeton) and *Mesaphorura* spp. increased as the summer progressed. *Isotomurus palustris* (Müller) was an early coloniser of decomposing oak leaves, while *Isotoma notabilis* Schäffer was a late coloniser. The significance of the findings is discussed with respect to current EU guidelines on the safe metal loading of sludge and soil.

Key words: Collembola, heavy metals, organic waste, fertiliser, succession, litterbags

Introduction

The Commission of the European Communities Directive concerning urban wastewater treatment, stipulated that from 1998 the disposal of sewage sludge at sea was to be no longer permitted (CEC 1991). As this was one of the principal methods of disposal in the UK (Smith 1996), alternative methods must now be found. In comparison to disposal methods such as incineration and landfill, using sewage sludge as an agricultural fertiliser not only provides a cheap method of disposal, but also a more environmentally friendly method. It has therefore been predicted that in the next decade the agricultural recycling of sewage sludge will become more widespread (Rund 1995).

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It is well documented that the increased nutrient levels that accompanies sludge application favours invertebrates, and the abundance of Collembola (Lübben 1989), Carabidae (Larsen et al. 1996), earthworms (Cuendet & Ducommun 1990), soil nematodes (Larink et al. 1990) and soil mites (Glockemann & Larink 1989) are all promoted by sludge application. The addition of sewage sludge to agricultural land can, however, be problematic as a consequence of some sludges containing significant quantities of pollutants such as polynuclear aromatic hydrocarbons (PAH's), polychlorinated biphenyls (PCB's) and heavy metals. Heavy metals are particularly problematic as they are persistent and can therefore accumulate in the soil to potentially toxic levels in areas of long-term application (Aitken et al. 1995). It is likely that such elevated metal levels resulting from long-term use will influence the invertebrate community.

Previous field studies have indicated that heavy metal contaminated sludge does not affect the overall abundance of gamasid mites (Glockemann & Larink 1989), euedaphic Collembola (Lübben 1989) or epigeal Collembola (Bruce et al. 1997), but can affect their population structure. Some collembolan species (e.g. *Folsomia candida* Willem) have been shown to prefer amended plots (i.e. those receiving sludge artificially contaminated by zinc, cadmium, copper, nickel, chromium and lead salts), while other species (e.g. *Isotoma notabilis*) preferred untreated plots (Lübben 1989). It would therefore appear, at least for Collembola and Acarina, that heavy metals have the potential to alter the species composition in subtle ways.

At present, application is controlled by regulations governing the safe metal loadings of sludge and soil (CEC 1986; CEC 1991). The influence of heavy metals in sewage sludge on insect communities has received little attention in contrast to the many studies on soil micro-organisms (Smith 1996) and more research is therefore required to ascertain that maximum metal levels set by current CEC legislation are adequate to protect invertebrates. As euedaphic and hemiedaphic Collembola live in close association with the soil micro-flora and fauna, it is likely that they will give an earlier indication of ecosystem disturbance than, for example, predatory groups (i.e. Araneae). Furthermore, as a consequence of their small size they are particularly useful to use as monitors in small plot trials were the use of larger more mobile invertebrates (i.e. Coleoptera) would be inappropriate.

The aim of this study was to investigate the effects of several different sewage sludges (including digested, undigested and metal-rich sludges) on euedaphic and hemiedaphic Collembola. This research was conducted as part of a larger scale experiment and consequently it was necessary to select a sampling method that did not unduly disrupt the experimental plots. Litterbags were therefore used to provide a sampling method that did not disturb the surrounding soil and also to enable seasonal and successional effects to be studied.

Materials and Methods

Study Area and Experimental Design

The study site was situated on a sandy clay loam (21 % clay; 2.6 % organic carbon) at SAC Auchincruive, Scotland.

This research was conducted as part of a larger experiment instigated in 1994. The larger experiment consisted of a total of 69 plots (each 6 m × 8 m) established across three blocks. Within each block, 23 sludge treatments were allocated to plots at random. Sludge was applied in the form of solid sludge cake to the experimental plots in August 1994 and incorporated into the soil to a depth of 25 cm. Each plot was surrounded by a 1.2 m permanent strip of grass to prevent transfer of soil between plots during cultivation processes. After incorporation, all plots were sown with Italian ryegrass (*Lolium multiflorum*).

Collembolan populations were monitored across five of these treatments each of which had three replicas (total of 15 plots). The treatments studied were: no sludge control, uncontaminated (i.e. low levels of heavy metals) sludge (digested), uncontaminated sludge (undigested), zinc-rich sludge (digested) and copper-rich sludge (undigested). See Table 1 for treatments and metal concentrations. The me-

Table 1. Treatments and mean metal concentration of sludge and soil

Treatment	*Mean weight of sludge applied in 1995*	*Mean sludge concentration (1994)*	*Mean soil concentration (1995)*
Control		Not applicable	83.9 mg Zn kg^{-1} soil 22.2 mg Cu kg^{-1} soil
Digested uncontaminated sludge	34 tonnes dry solids/ha	849 mg Zn kg^{-1} sludge 693 mg Cu kg^{-1} sludge	91.0 mg Zn kg^{-1} soil 30.3 mg Cu kg^{-1} soil
Undigested uncontaminated sludge	28 tonnes dry solids/ha	532 mg Zn kg^{-1} sludge 456 mg Cu kg^{-1} sludge	87.9 mg Zn kg^{-1} soil 27.1 mg Cu kg^{-1} soil
Zinc-rich digested sludge	41 tonnes dry solids/ha	5,238 mg Zn kg^{-1} sludge	180 mg Zn kg^{-1} soil
Copper-rich undigested sludge	87 tonnes dry solids/ha	4,331 mg Cu kg^{-1} sludge	69 mg Cu kg^{-1} soil

tal-rich sludges were derived from sewage treatment works with naturally high inputs of the respective metal. The organic matter input was standardised across sludge treatments and the pH was maintained at 6. Each year the grass on all plots was cut twice for silage. In 1995, grass cutting occurred on 24 May and 17 July.

Sampling Techniques

Litterbags were constructed from 15 cm x 15 cm squares of nylon mesh (mesh size 1 mm) and were filled with 2.50 grams of oven dried oak leaves (*Quercus robur*). The litterbags were rehydrated before burial to encourage colonisation. On 6 December 1994, 15 litterbags were buried at random to a depth of 2–5 cm in the selected plots (total of 225 litterbags). Litterbags were not established within a distance of 1m from the edge of the plot to eliminate any edge effect. Five litterbags from each plot were selected at random and collected after 10, 20 and 30 weeks (see Figure 1). A further five litterbags were buried in each plot on 16 May 1995, and these were lifted after 10 weeks. The experiment was designed to provide information on succession (by comparing the 10, 20 and 30 week litterbags) and season (by comparing the two sets of litterbags buried for 10 weeks).

Cylinder extractors similar to those described by MacFadyen (1961) were used to extract Collembola. While MacFadyen (1961) used a water bath to maximise the extraction gradient, in this study extraction was improved by placing the collection vials on ice bags which were renewed daily. This is likely to have improved extraction not only by increasing the temperature gradient, but also by preventing the alcohol from evaporating (alcohol fumes can repel Collembola: MacFadyen (1961)). Extraction was conducted over a period of six days. Following extraction Collembola were mounted in Hoyer's medium and identified under a light microscope (magnification x 400–1,000) following Fjellberg (1980), Gisin (1960) and Gough (1977). For the purpose of this research species were identified following the nomenclature of Fjellberg (1980).

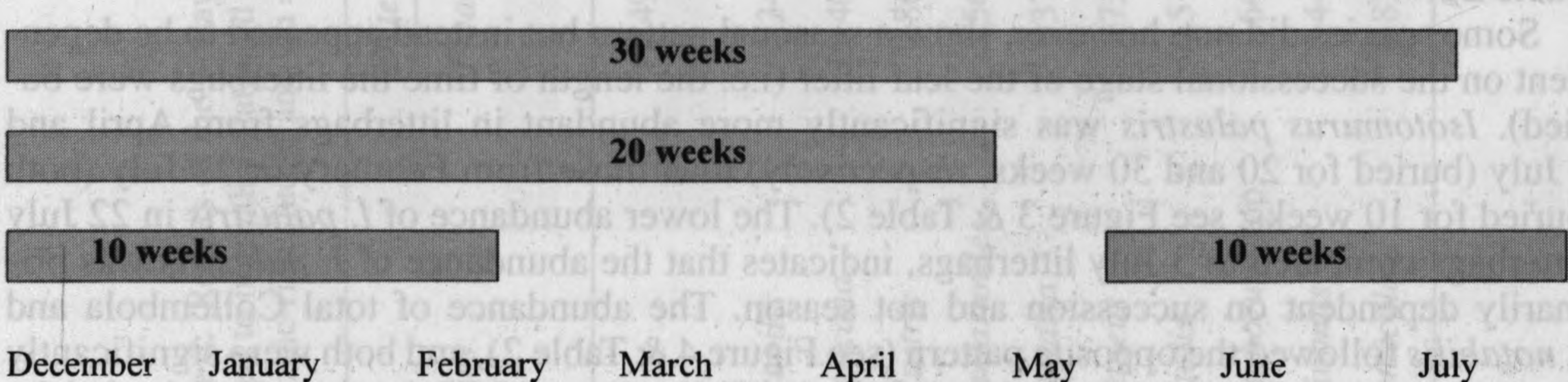

Fig. 1. Burial and lifting times for litterbags

Statistical Analysis

The Collembola extracted from the five samples taken from each plot on a particular sampling date were pooled to obtain an overall indication of collembolan community structure for each plot on a specific sampling date. The pooled data were used in subsequent statistical analysis.

Analysis of Variances. Variance and means ratios were checked prior to all analyses to determine if species abundances were normally distributed. Most species were not normally distributed and square root transformation was found to be more effective at normalising the data (for the majority of species) than the more common log transformation. It was deemed inappropriate to use different transformations for different species and it was therefore necessary to use a standard transformation that worked for the majority of the species (i.e. the square root transformation). It was, however, recognised that after transformation the variances for some species were still heterogeneous and consequently the significance value was set at 0.01 to avoid wrongly rejecting the null hypothesis (Day & Quinn 1989).

Three-way Analysis of Variance (ANOVA), without replication, were performed (on the square root transformed data) to test for date/succession, block and treatment effects. Examination of the data prior to analysis indicated that interaction did not occur and it was therefore possible to perform the tests without replication hence enabling block effects to be investigated. ANOVAs were performed on the abundance of total Collembola and frequently occurring species (i.e. those occurring in over 50% of samples). The Tukey multiple comparison test was applied to locate significant differences identified by ANOVA.

Diversity. The following measures of diversity were calculated: S (number of species) to measure species richness, d (Berger & Parker 1970) to measure evenness and N_2 (Hill 1973) and α (Fisher et al. 1943) to measure overall diversity. It is thought that by combining indices the most accurate picture of diversity is obtained (Magurran 1988). Three-way ANOVAs (followed by Tukey's multiple comparison tests) were conducted (on the square root transformed data) to test for the effect of treatment, block and date on diversity.

Results

General information

A total of 14,829 individuals, from 26 species, were obtained from litterbags collected between 14 February and 22 July 1995. Only ten of these species occurred in sufficient numbers to allow standard statistical analyses and therefore the remainder of this paper is restricted to a consideration of these.

Species composition and date

From Figure 2 and Table 2 it can be seen that abundance is primarily influenced by season in *L. cyaneus* and *H. nitidis* with both species increasing as the summer progressed. Both were significantly more abundant in July samples than February or April ones, and they were also more abundant in 22 July samples than 3 July samples. A simple successional effect can be discounted as the litterbags buried in February and 22 July were both exposed for a 10 week period yet differed most with respect to the abundance of these two species. *Mesaphorura* spp. (consisting of *M. macrochaeta* Rusek and *M. hylophila* Rusek) followed a similar pattern with the highest abundance being found in litterbags collected on 22 July (see Figure 2 & Table 2).

Some species did not, however, show a seasonal pattern but instead appeared to be dependent on the successional stage of the leaf litter (i.e. the length of time the litterbags were buried). *Isotomurus palustris* was significantly more abundant in litterbags from April and 3 July (buried for 20 and 30 weeks, respectively) than those from February or 22 July (both buried for 10 weeks: see Figure 3 & Table 2). The lower abundance of *I. palustris* in 22 July litterbags compared to 3 July litterbags, indicates that the abundance of *I. palustris* was primarily dependent on succession and not season. The abundance of total Collembola and *I. notabilis* followed the opposite pattern (see Figure 4 & Table 2), and both were significantly more abundant in litterbags buried for 10 weeks (February and 22 July) than those buried for 30 weeks (3 July). The abundance of *I. anglicana* appeared to be dependant on both season

Table 2. Results of the 3-way ANOVAs followed by Tukey Tests for dominant species (probability for Tukey Tests set at 0.01). Data were square root transformed to normalise data and to homogenise variances. The codes for the treatments are as follows: C = control, D = digested sludge, U = undigested sludge, Zn = zinc-rich sludge and Cu = copper-rich sludge. As no block effect was found, this information is omitted from the table

	Date			*Treatment*		
Species	*F-value*	*Probability* $df = 3, 24$ $n = 15$	*Location of differences* $df = 24, k = 4$	*F-value*	*Probability* $df = 4, 24$ $n = 12$	*Location of difference* $df = 24, k = 5$
Total	10.49	< 0.001	22 July & February > 3 July 22 July > April	1.87	N.S.	–
I. notabilis	6.34	0.001	February & 22 July > 3 July	1.23	N.S.	–
I. anglicana	19.40	< 0.001	3 July > 22 July > April > February	2.53	0.05	Zn > All, Cu > U, D & C
I. palustris	7.86	< 0.001	3 July > April > February & 22 July	0.25	N.S.	–
F. fimetarioides	2.56	N.S.	–	0.31	N.S.	–
F. fimetaria	1.37	N.S.	–	1.76	N.S.	–
L. cyaneus	7.73	< 0.001	22 July > 3 July > February & April	0.74	N.S.	–
H. nitidis	140.51	< 0.001	22 July > 3 July > February & April	1.78	N.S.	–
Mesaphorura spp.	11.64	< 0.001	22 July > 3 July & April > February	2.68	< 0.05	C, D, U > Zn & Cu
N. minimus	2.44	N.S.	–	2.75	< 0.05	C > D, All > Zn, Cu > D & U
C. denticulata	6.83	< 0.001	February & 22 July > April	1096	N.S.	–

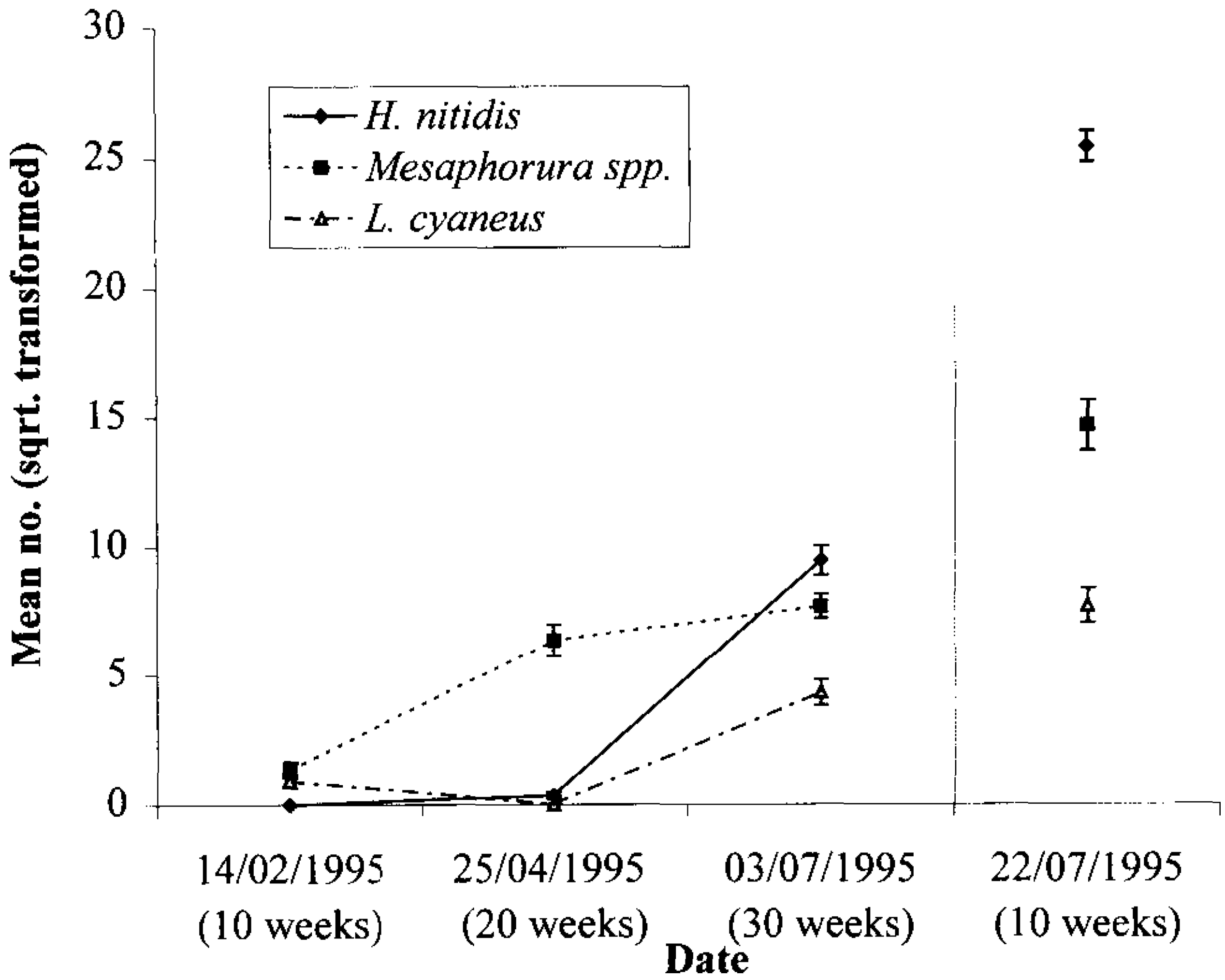

Fig. 2. Effect of date on abundance of *H. nitidis*, *L. cyaneus* and *Mesaphorura* spp. irrespective of treatment. The results to the left of the dividing line were obtained from litterbags buried on 6 December 1994 and lifted after 10, 20 or 30 weeks, while the results to the right of the line were obtained from litterbags buried on 16 May 1995 and lifted after 10 weeks. Error bars show standard deviations

and succession. This species had a higher abundance in April litterbags (20 weeks) than February litterbags (10 weeks), and also had a higher abundance in 3 July litterbags (30 weeks) than April litterbags (20 weeks: see Figure 4 & Table 3). Like *I. palustris*, this species was less abundant in litterbags from 22 July (10 weeks) than 3 July (30 weeks), but unlike *I. palustris*, the abundance on 22 July was greater than in February or April. This suggests that changes in the abundance of *I. anglicana* were not just the consequence of succession but also of season.

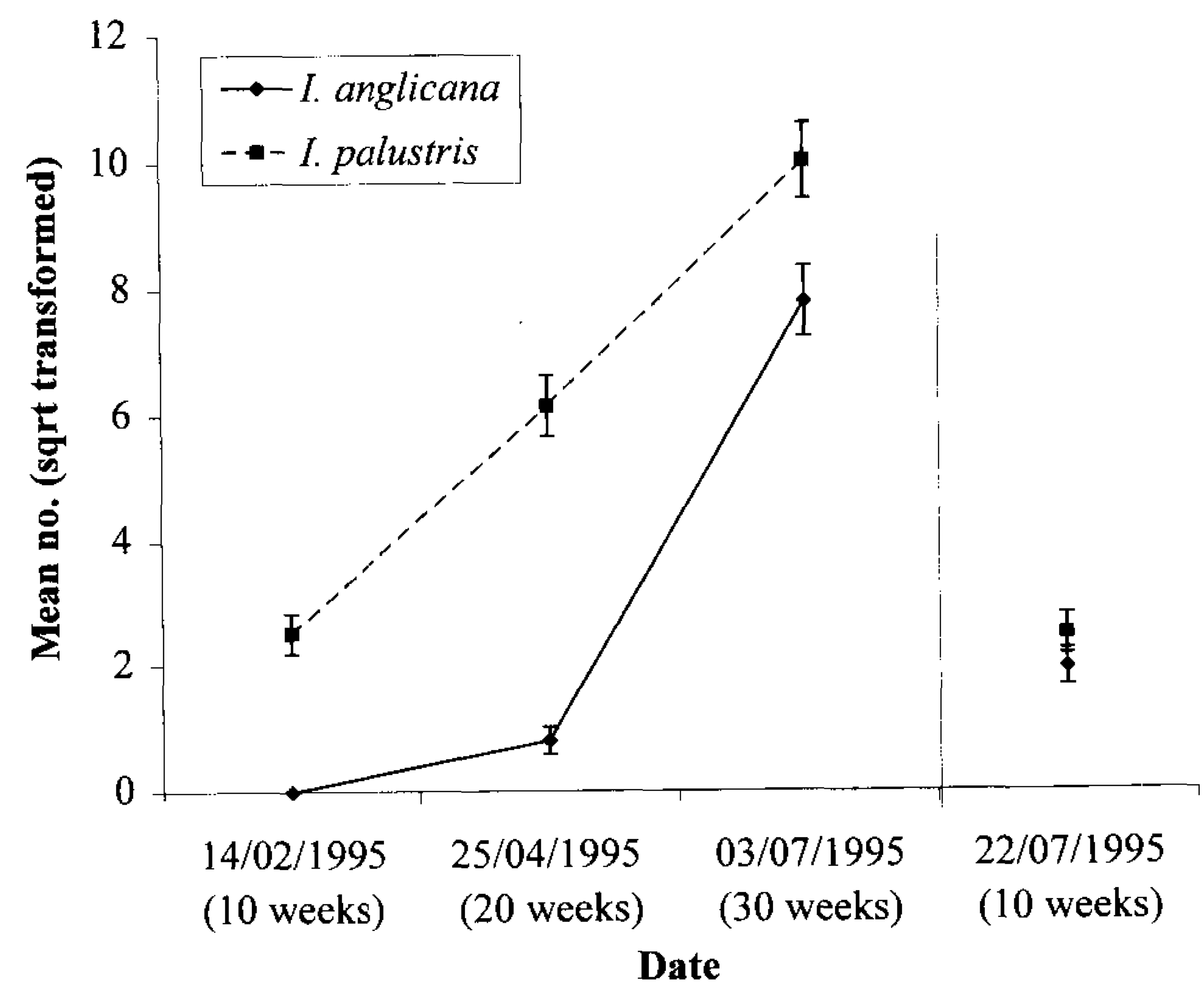

Fig. 3. Effect of date on abundance of *I. anglicana* and *I. palustris* irrespective of treatment. The results to the left of the dividing line were obtained from litterbags buried on 6 December 1994 and lifted after 10, 20 or 30 weeks, while the results to the right of the line were obtained from litterbags buried on 16 May 1995 and lifted after 10 weeks. Error bars show standard deviations

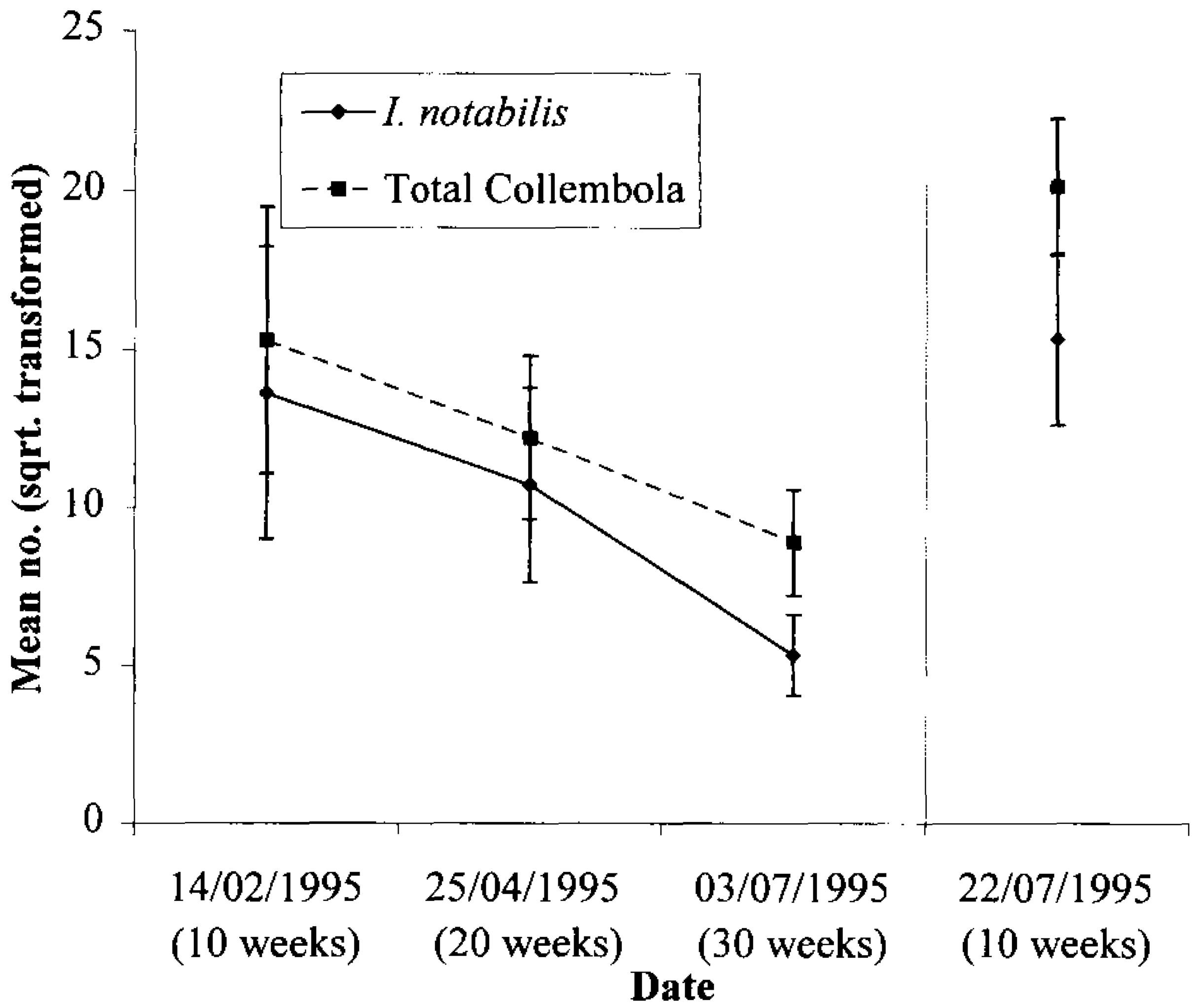

Fig. 4. Effect of date on abundance of total Collembola and *I. notabilis* irrespective of treatment. The results to the left of the dividing line were obtained from litterbags buried on 6 December 1994 and lifted after 10, 20 or 30 weeks, while the results to the right of the line were obtained from litterbags buried on 16 May 1995 and lifted after 10 weeks. Error bars show standard deviations

Species composition and treatment

Contrary to the findings of other studies (Höller-Land 1959; Lübben 1989) the application of sewage sludge did not significantly increase the abundance of Collembola. Effects of sludge were however, found at the species level and the abundance of *Neelus minimus, Mesaphorura* spp. and *I. anglicana* were influenced by sludge application (see Table 2 & Figure 5). *Isotoma*

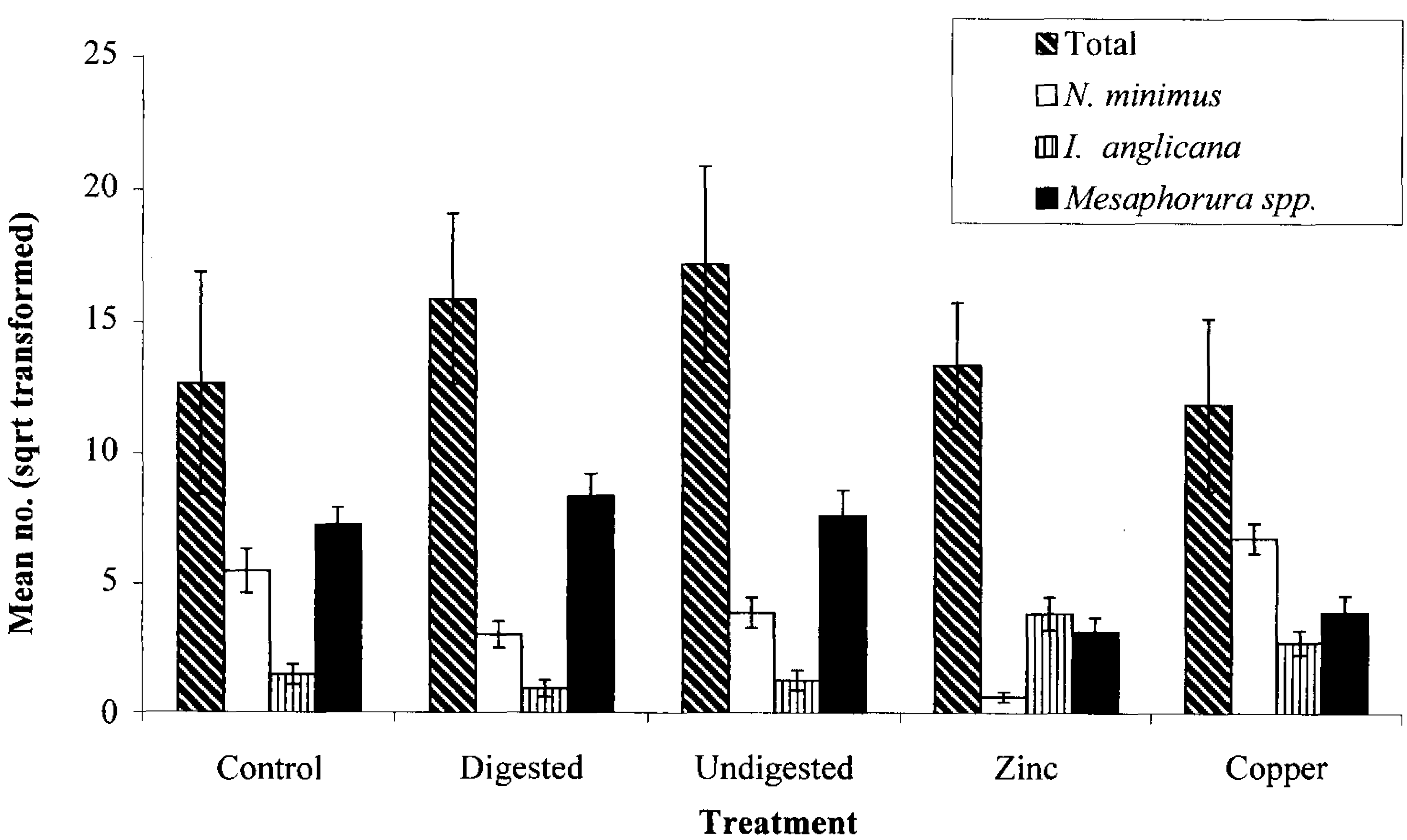

Fig. 5. Effect of treatment on Collembola irrespective of date. Error bars show standard deviations

anglicana was more abundant in zinc and copper-rich plots than undigested, digested or control plots. *Mesaphorura* spp., on the other hand, were less abundant in zinc and copper-rich plots than undigested, digested or control plots. *Neelus minimus* had a higher abundance in copper-rich plots but a lower abundance in zinc-rich plots than in plots receiving uncontaminated sludge (undigested or digested). The abundance of *N. minimus* was also lower in plots receiving digested sludge than in control plots, suggesting adverse affects of digested sludge. These results must, however, be treated with caution as the variances were heterogeneous which can increase the probability of finding an effect of treatment when none is present (Day & Quinn 1989). Differences between treatments were significant at a probability of 0.01 for the Tukey tests suggesting that treatment effects were real.

Diversity

The large input of nutrients accompanying sludge application may be expected to favour some species over others resulting in a less even community. This did not, however, appear to be the case in this study and diversity was not affected by sludge application. Effects of date were however apparent. Species richness (S) showed a seasonal pattern with July samples having more species than February or April samples. This was likely to be the result of season rather than succession because the largest difference in species richness occurred between the 14 February and 22 July samples (both buried for 10 weeks: see Table 3). Both overall diversity measures (α and N_2) were higher in litterbags from 3 July (30 weeks) than those from February (10 weeks), April (20 weeks) or 22 July (10 weeks) suggesting a successional effect. The dominance (d) was less (indicating a more even community) on 3 July (30 weeks) than in February (10 weeks) or April (20 weeks), but not 22 July (10 weeks). This indicates both season and succession affected dominance.

Table 3. ANOVAs followed by Tukey Tests performed on diversity indices. Indices were square root transformed prior to analysis to normalise data and to homogenise variances. Mean values of diversity indices are given for each sampling date with standard deviations shown below

Diversity Index	*F-value*	*Probability df=3, 24 n=15*	*February*	*April*	*3 July*	*22 July*	*Location of difference df = 24, k = 4*
Species Richness (S)	20.90	<0.001	2.28 ±0.41	2.60 ±0.43	3.04 ±0.32	3.25 ±0.23	3 & 22 July > April & February
Berger-Parker (d)	15.85	<0.001	0.89 ±0.12	0.88 ±0.12	0.65 ±0.08	0.77 ±0.11	February & April > 3 July
Hill's (N_2)	18.40	<0.001	1.26 ±0.28	1.31 ±0.35	1.99 ±0.28	1.58 ±0.29	3 July > February, April & 22 July 22 July > February
Fisher's (α)	18.05	<0.001	1.07 ±0.32	1.26 ±0.24	1.75 ±0.32	1.42 ±0.15	3 July > February, April & 22 July 22 July > February

Discussion

With respect to sampling date collembolan species fell into three main categories: species that are dependent on season (e.g. *L. cyaneus, H. nitidis* and *Mesaphorura* spp.); species that are dependent on the successional stage of the leaf litter (e.g. *I. notabilis* and *I. palustris*); and species unaffected by season and succession (e.g. *F. fimetarioides, F. fimetaria* and *N. minimus*). Other litterbag studies have also found both seasonal and successional effects on populations of Acari and Collembola (Anderson 1975; Bolger 1985).

Goulden (1969) proposed that as leaf material decomposes, the diversity of organisms involved in decomposition progress through a series of developmental phases. In the first phase, the colonisation phase, species diversity increases as species rapidly immigrate into the new habitat. In the second phase, the common species become less common and the rare species less rare (the evenness of the community increases). In the third phase, rare species continue to colonise the habitat until it is fully saturated, and finally in the fourth phase, the diversity is degraded to a few abundant species adapted to the more unfavourable environment of humus. Field studies using litterbags have generally supported Goulden's theory (Anderson 1975; Bolger 1985; Siepel 1990). In this study litterbags were rapidly colonised within the first ten weeks of burial which is indicative of Goulden's (1969) colonisation phase. The evenness of the community increased as the succession of leaf litter in the litterbags progressed, as predicted by Goulden's phase two. However as species richness was predominately determined by season, it was difficult to determine if phase three had occurred and there was no evidence that phase four was reached.

The addition of sewage sludge usually favours invertebrates and this is thought to be primarily the result of an increase in food (Pimentel & Warneke 1989). Contrary to the findings of other studies (Höller-Land 1959; Lübben 1989), this study found no increase in collembolan abundance following sludge application. Anaerobic conditions and high ammonia levels can occur following sludge application and these may have prevented the Collembola from benefiting from the increased nutrient levels. Gusenleitner (1959) found that the application of liquid-manure adversely affected collembolan diversity. Heavy metals in sewage sludge may also be expected to decrease diversity (although this was not the case in this study site) as heavy metals have previously been found to adversely affect diversity (Bengtsson et al. 1985).

Mesaphorura spp. were adversely affected by copper and zinc-rich sludge and *N. minimus* was adversely affected by zinc-rich sludge, at soil metal concentrations well below the levels deemed safe by current UK legislation (i.e. 300 mg Zn kg^{-1} soil and 140 mg Cu kg^{-1} soil). Although laboratory ecotoxicological studies have not been conducted on either of these species, they have been conducted on the euedaphic species *F. candida* (Smit & Van Gestel 1996; Van Gestel & Hensbergen 1997) and *Folsomia fimetaria* Linné (Scott-Fordsmand et al. 1997). Smit & Van Gestel (1996) reported EC50 values for zinc of 185 to 348 mg Zn kg^{-1} soil for reproduction in *F. candida*, while Van Gestel & Hensbergen (1997) reported higher values of 626 to 683 mg Zn kg^{-1} soil. EC50 values for copper of 113 mg Cu kg^{-1} soil and EC10 values of 38 mg Cu kg^{-1} soil were reported for reproduction in *F. fimentaria* (Scott-Fordsmand et al. 1997). Despite EC50 levels differing considerably between studies and species (Scott-Fordsmand et al. 1997), they provide useful information on what metal concentrations can directly affect Collembola under standardised conditions. The soil concentration in the zinc-rich and copper-rich plots (i.e. 180 mg Zn kg^{-1} soil and 69 mg Cu kg^{-1} respectively), are therefore similar to levels where reproduction has been adversely affected in the laboratory. It is therefore possible that the metals directly affected reproduction in *N. minimus* and *Mesaphorura* spp. resulting in the lower densities observed in plots receiving metal-rich sludge.

Copper and zinc may also have indirectly affected Collembola through altering the microbial composition (and hence food for the Collembola) and the abundance of predators and competitors. *Folsomia fimetarioides* (Axelsson) was found to be more abundant in polluted areas than *Isotomiella minor* (Schäffer) and this was thought to be a consequence of *F. fimetarioides* preferring metal tolerant fungi while *I. minor* preferred susceptible species (Tranvik & Eijsackers 1989). Furthermore *F. fimetarioides* was able avoid polluted food, an ability *I. minor* did not share (Tranvik & Eijsackers 1989). *Mesaphorura* spp. and *N. minimus* may therefore be particularly sensitive because they are unable to avoid polluted food or their preferred fungal species are metal sensitive.

The susceptibility of *Mesaphorura* spp. to metal-rich sludge is surprising as *M. krausbaueri* was favoured in areas polluted by copper containing fungicides (Filser et al. 1995). Furthermore, *Mesaphorura* spp. occurred in their highest abundance in plots treated with

metal-rich sludge in a study by Lübben (1989). Discrepancies between previous studies and this study may be due to species specific differences in metal susceptibility existing within the genus *Mesaphorura*. It is well documented that collembolan species differ in their susceptibility to metal pollution (Filser et al. 1995; Tranvik & Eijsackers 1989; Posthuma & Van Straalen 1993).

As heavy metals decrease with soil depth (Bengtsson & Rundgren 1988), it is possible that *Mesaphorura* spp. and *N. minimus* (being strictly soil species) did not actually decrease in abundance, but instead migrated down the soil profile to avoid high metal levels. Bengtsson & Rundgren (1988) found that euedaphic Collembola migrated to greater soil depths (up to a depth of 10 cm) in metal polluted areas, and the migration of soil Collembola to avoid adverse weather conditions is well documented (Hale 1967; Marshall 1974). However, as the sewage sludge (and therefore the heavy metals) were incorporated into the soil up to a depth of 25 cm, it is unlikely that the Collembola were able to follow a concentration gradient to less polluted areas.

Isotoma anglicana was promoted by the addition of copper-rich and zinc-rich sludge, while *N. minimus* was promoted by the addition of copper-rich sludge. Zinc and copper are essential micro-nutrients (Hopkin 1989) thought to be required in trace quantities by Collembola (Bengtsson et al. 1983). It is possible that *I. anglicana* and *N. minimus* were deficient in these metals prior to sludge application and were therefore favoured by the increased metal levels following application. Low levels of copper have been found to increase the growth rate of *Onychiurus armatus* (Tullberg) in the laboratory (Bengtsson et al. 1983), and the collembolan density in the vicinity of Gusum brass mill was higher at low levels of copper and zinc pollution than at unpolluted areas (Bengtsson & Rundgren 1988). The increase in abundance at low metal levels may also be the consequence of these species possessing metal-sensitive gut parasites, or of predators and/or competitors being more susceptible to zinc and copper. *Isotoma viridis* Bourlet, which is closely related to *I. anglicana*, has previously been found to be sensitive to the presence of zinc in sewage sludge (Bruce et al. 1997).

It is important to note that as a consequence of the sludges arising from different sewage treatment works, other differences between sludges existed and these may have affected the collembolan community. For example, it is possible that the metal-rich sludges, being derived from more industrial areas, had higher levels of organic pollutants such as PAH's and PCB's. Metals are, however, generally less available in sludges derived from treatment works with high metal inputs than in artificially contaminated sludge (Smith 1996) and the most accurate risk assessment of metal-rich sludge is therefore obtained using naturally contaminated sludge.

From the results of this study it would appear that while the total collembolan abundance was not influenced by the addition of metal-rich sludge, their population structure was altered at metal levels currently deemed safe by CEC legislation (1986; 1991). Through altering the soil ecosystem, soil processes such as decomposition and mineralisation may also be affected and it is therefore feasible that this could counteract the fertilising potential of the sludge. It is, however, not possible to comment at this stage as to whether current legislation is sufficient to ensure that the soil ecosystem is adequately protected. Further work is therefore required before the full implications of the observed changes to population structure can be ascertained.

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